

## The *Drosophila pinicola* Species Group

(Diptera: Drosophilidae)

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Two Western North American species, i.e., *D. flavopinicola* Wheeler and *D. pinicola* Sturtevant, constitute the *Drosophila pinicola* species group. Sturtevant (1942) in his analysis of the evolution and phylogeny of *Drosophila* concluded (1) that *D. pinicola* belongs to the subgenus *Drosophila* with its closest relatives being members of the *D. virilis* species group, and (2) that *pinicola* is also closely related to both the genus *Scaptomyza* and the subgenus *Sophophora* of *Drosophila*. Since he assumed that *Scaptomyza* and *Drosophila* were coordinate genera, he regarded *pinicola* to be a "primitive type." Patterson and Stone (1952) accepted Sturtevant's interpretation. Throckmorton (1962, 1966 and 1968) showed that *Scaptomyza* is not coordinate with *Drosophila* but rather that *Scaptomyza* is an "exgroup genus derived from one of the lineages within the genus *Drosophila*." Throckmorton concluded that *pinicola* is a relative of *Scaptomyza* but he excluded *pinicola* from being considered as a primitive member of the genus *Drosophila*.

Investigations of the endemic Hawaiian Drosophilidae (Hardy, 1965; Throckmorton, 1966; Spieth 1966; Carson et al. 1970) uncovered a surprising degree of overlap in various characteristics of the endemic Hawaiian drosophiloids and scaptomyzoids. A resultant possible conclusion from such data is that the genus *Scaptomyza* arose on the Hawaiian islands from the same ancestral immigrant stock that also gave rise to the endemic drosophiloids. Further, the characteristics of the Hawaiian *Drosophila* species are such that their ancestor could not have been derived from any known South Pacific drosophiloid group. Some area of the North Pacific appears more likely to have been the ancestral home of the original introduction that gave rise to Hawaiian drosophiloid fauna. Additionally, a recent study (McDonald, Heed and Miranda, 1974) which compares the larval leaf-feeding habits of species of Lauxaniidae from the north coast of California with Hawaiian leaf-breeding *Drosophila* elucidates the ecological similarities between the two areas.

Because of the phylogenetic placement of the *pinicola* subgroup, its geographical distribution and the ecological similarities, it therefore seems appropriate to investigate its possible relationship to the Hawaiian fauna.

#### DISTRIBUTION

The exact geographical limits of the distributions of the two species have not been determined, but present data indicate that they are allopatrically distributed in the coniferous or mixed coniferous-hardwood forests of California, Oregon and Washington. *D. pinicola* is known to range from Mt. San Jacinto in Southern California, northward on both sides of the San Joaquin and Sacramento valleys to Yosemite National Park (Mather) on the eastern side and to St. Helena, California, on the western side. Neither species has been collected east of the Sierra Nevada Range in California or the Cascade Range in Washington and Oregon. *D. flavopinicola*, originally collected by Wheeler and Heed in 1951, has been found only in the coastal areas extending from Trinidad, California, to the Olympic National Forest (Sequim) in Washington.

#### NATURAL HISTORY

*Drosophila pinicola* has been reared from field-collected mushrooms (Dobzhansky, personal communication). We have reared *D. flavopinicola* from several species of soft bodied mushrooms, especially species of *Amanita*. In the Trinidad, California, area three drosophilid species (*D. flavopinicola*, *D. melanderi* Sturtevant, and *D. subquinaria* Spencer) utilize the same individual fungi (mushrooms) for ovipositional substrates. Field-collected individual mushrooms produced in the laboratory numerous adults of all three of the *Drosophila* species as well as a species of Psychodidae, two species of Tipuloidea and two additional species of Acalypterate diptera. How these eight species are able successfully to partition the resources of a single fungal body has not been determined.

None of the three (*flavopinicola*, *melanderi* and *subquinaria*) appears to have a large population of adults in the field, even when suitable mushrooms are abundant. At the Trinidad sites direct observation, sweeping over mushrooms and baiting with bananas all indicate that *subquinaria* is the rarest of the three and *flavopinicola* the most abundant. At Mather, California, during the late spring and early summer when mushrooms are present in numbers, *pinicola* is quite abundant but it is rare at other seasons.

*D. melanderi* males assume courtship stations on the upper surface

of mushroom caps. Highly pugnacious, they vigorously defend the area surrounding them. As a result, usually one and never more than two *melanderi* males have been observed on a single mushroom cap. If another drosophilid individual enters the defended area, the male will immediately approach and attempt to court.

*D. flavopinicola* males apparently never utilize the upper surface of the mushroom cap, but, rather, sit on the substrate around the base of the mushroom, perhaps on the mushroom stem. The male courtship displays thus seem to occur primarily on the ground at the base of the mushroom rather than on the mushroom proper. The courtship behavior of neither *D. subquinaria* nor *D. pinicola* was observed in the field.

In the laboratory both *flavopinicola* and *pinicola* prefer to oviposit in the commercial mushroom *Agaricus disporus* rather than in cornmeal or banana media. Larvae hatched from eggs oviposited in *A. disporus* complete their development and produce normal adults. The adults, however, cannot be maintained solely on *A. disporus* and must have access also to a carbohydrate rich food source such as the standard *Drosophila* media. Significantly, Sturtevant (1942) suggested that the adults of *pinicola* are sap feeders since he observed them on moist spots on the trunks of *Sequoiadendron giganteum* (Lindl.).

The mature larvae of *flavopinicola* and *pinicola* avoid pupating in or on the surface of their food substrate. Typically they pupate either in the cotton plug of the rearing vial or crawl through the plug and escape from the rearing bottle. Removal of the cotton plug and placement of the open rearing vial in a larger jar which has a layer of moist sand on its bottom results in the mature larvae leaving the rearing vial and pupating on the surface of the sand.

Similarly, the larvae which develop in a field collected mushroom which has been placed in a "sand jar" leave the mushroom when mature and pupate on the sand some distance from the remains of the mushroom.

In the laboratory *flavopinicola* and *pinicola* will oviposit in any portion of a mushroom, although they prefer the stem and cap surfaces and tend to avoid the gills. *D. subquinaria* exhibits similar behavior but *melanderi* refuses to oviposit in the gill area and prefers the surface of the mushroom cap.

Sturtevant (1942) noted that *pinicola* was difficult to maintain under laboratory conditions and must be kept at low temperatures. The reason is that the female oocytes of both species will not develop unless the individuals are kept at a temperature below 18–19°C. The adults

can remain apparently healthy at higher temperatures, e.g., at 22–25°C, and at least some of the males will develop viable sperm. Occasionally on old female will become inseminated even though her ovaries show no sign of development. If individuals which are sterile because of “high” temperature are then placed under 18°C temperature, the females will within 7–10 days develop mature fertile ova.

It thus appears that *flavopinicola* and *pinicola* are fungivorous species that are constrained to live in areas where the females are able to find micro niches having temperatures lower than 18°C for relatively prolonged periods of time. The adults must be able to find both fungus in which the females can oviposit and an additional food substrate for themselves.

#### LIFE CYCLE

Both *flavopinicola* and *pinicola* reach maturity slowly and there is considerable inter-individual variation between flies reared and aged together. *D. flavopinicola* females reared and maintained at  $18^{\circ}\text{C} \pm 1.5$  begin to deposit eggs when they are 20–22 days old. *D. pinicola* matures about five days faster than does *flavopinicola*. Embryonic, larval and pupal development takes about 20 days for *pinicola* and 25 days for *flavopinicola*, thus resulting in generation times of about 35 and 45 days, respectively.

#### MORPHOLOGY

Wheeler (1954) noted that *flavopinicola* adults differ from *pinicola* mainly in their larger size, more yellowish body color and more highly pigmented wings. Thus *pinicola* appears blackish with a brownish tinge, and has clear wings except for a faint melanistic cloud on the posterior cross vein. It varies from 2.5–3.0 mm in body length and from 3.0 to 3.5 mm in wing length. *D. flavopinicola* appears brownish with a yellowish tinge, and has strong melanistic clouds on both cross wings and in the costa cell. The entire wing is also suffused with a brownish tinge. It varies from 3.5 to 4.5 mm in body length and from 4 to 5 mm in wing length. *D. pinicola* is therefore a medium sized species while *flavopinicola* is one of the largest drosophiloids found in North America. The eggs, larvae, pupae, the male genitalia, the ovipositors and the female ventral receptacles and dorsal spermatheca of the two species are also essentially identical except for size.

#### KARYOLOGY

The larval metaphase chromosomes of *D. flavopinicola* were described by Clayton and Ward (1954) as 5 pairs of rods and one pair



of dots. The X and Y chromosomes could not be distinguished. We examined one larval brain smear from Trinidad, California (sex undetermined) and found the same configuration as in Plate 1 of Clayton and Ward with the exception that the sex chromosomes were longer, about 3 times the length of the autosomes and had satellites. The microchromosomes were minute. The larval polytene chromosomes showed 5 arms plus a dot with much diffuse centromeric heterochromatin.

The metaphase chromosomes of *D. pinicola* were described by E. Novitski in Sturtevant (1942) as follows: "Three pairs: X is J-shaped, Y is V-shaped, each arm about as long as the long arm of the X. There are two pairs of autosomes, a V and a rod, each of the three arms concerned being about the length of the short arms of the X." We have examined two male and two female larval ganglion cells (Stock M-72-12) and concur with the description above but with the following additions: (1) there is a pair of very small microchromosomes (dots); (2) the long arm of the X is about 3 times the length of the arms of the autosomes, and (3) the Y chromosome has a secondary constriction in one of its arms. The larval polytene chromosomes show 5 arms plus a dot with much diffuse centromeric heterochromatin.

*D. pinicola* and *flavopinicola* have the following characteristics in common: double length X chromosomes probably due to the addition of heterochromatin, and very small dot chromosomes. The *pinicola* metaphase is derived by one autosomal fusion and an X-autosomal fusion.

#### BEHAVIOR

(1) *D. pinicola*: A sexually alert male moves about bobbing his abdomen up and down, then orients upon a nearby or passing female, approaches and strikes sharply against her body with one or both forelegs. He then moves quickly to the rear of the female, positions himself directly behind her and lunges forward, thrusts his head under her wings, curls the tip of this abdomen under and forward and attempts to make genitalic contact. At the same time he attempts to grasp the female's abdomen with his forelegs and to push her wings apart with his head. A receptive female allows the male to achieve intromission, mount, and spread her wings. During copulo all of the male's legs are engaged in grasping the female's body but 1 to 2 minutes before termination of the copulation the male releases his legs, falls backwards and assumes a motionless trance-like state. During this time the female kicks with her hind legs, attempting to break the genitalic union, and eventually the male abruptly withdraws.

A non-receptive female may kick or decamp but most frequently she elevates the tip of her abdomen and "extrudes" by exposing the articulating membranes surrounding the ovipositor and terminal sclerites.

Copulation is relatively long, averaging 15'11", with a maximum of 19'17" and a minimum of 12'40".

(2) *D. flavopinicola*: A sexually alert *flavopinicola* male bobs his abdomen up and down. The amplitude of movement is larger than that displayed by *pinicola* and is accompanied by small up and down movements of both wings. The male exudes an anal droplet and infrequently depresses the tip of his abdomen against the substrate and deposits the anal liquid on the substrate. Typically he moves about with short quick steps as he displays. Periodically he will cease bobbing, clean the tip of his abdomen with his hind legs and then uses the same legs to clean his wings. Presumably the anal droplet contains a volatile pheromone which is thus released into the atmosphere. Such low intensity display may continue for prolonged periods but usually it quickly and smoothly changes into a high intensity display which involves continuance of the abdominal bobbing accompanied by flicking both wings outward and upward in increasing amplitude until after several flicks the wing vanes attain about 45° outward and upward. Both vanes are then held immobile in this extended position. Simultaneously he depresses his head and elevates his abdomen until his longitudinal axis forms a 30°–40° angle with the substrate and the large genital claspers are fully opened. He then engages in "bounce-bobbing," i.e., the entire body is bounced up and down; with each bounce both extended wings are flicked 7°–10° further outward and at the same time the body is rotated slowly back and forth about 15° along its longitudinal axis. Five to six bounce-bobs occur while a single rotation cycle is completed from one side to the other and back. This complex display occurs in bouts of 1–2 second's duration and may be repeated many times. Between bouts the male often stamps rapidly against the substrate with one or both hind legs.

A *flavopinicola* male will spontaneously display in an observation cell and if a female moves into close proximity he orients upon her, moves to her rear, taps with his forelegs and lunges forward thrusting his head under her wings, simultaneously curling his abdominal tip forward. He then grasps the female with his forelegs, attempts to mount and achieve intromission. A receptive female allows the male to mount, achieve intromission and spread her wings. During copulation the male's legs are all used to grasp the female but he does not go into a trance at the end of the copulatory period. Rather, he quickly dismounts

without preliminary movement. A non-receptive female may kick, decamp, or elevate the tip of her abdomen and extrude. The duration of copulation is quite similar to that of *pinicola* with an average time of 16'04'', a maximum of 20'30'' and a minimum of 11'50''.

The courtship of the two species displays unique elements when compared to other continental species that have been studied.

Males of both species engage spontaneously in display activities. This is surprising since almost invariably *Drosophila* males orient, approach, tap with their forelegs and then engage in display actions (Bastock and Manning 1955; Spieth 1952). The display of *pinicola* is quite simple and consists simply of abdomen bobbing. The display of *flavopinicola* is more complex and ranges from abdomen bobbing, a low intensity display, similar to the courtship of the *pinicola* male to a complex high intensity display involving the entire body of the fly. The transition from low to high intensity is smoothly graduated. Significantly, the *flavopinicola* male will orient and attempt to mount and copulate regardless of the level of intensity of his display at the time the female is encountered.

The elements of mounting, intromission, copulation posture and dismounting behavior are essentially identical for the two species except that the *pinicola* male displayed the motionless trance-like state at the termination of copulation, whereas with *flavopinicola* this element was never observed.

#### DISCUSSION

Egg, larval, pupal and adult morphologies indicate that *flavopinicola* and *pinicola* are closely related. *D. flavopinicola* possesses the primitive *Drosophila* karyotype of five pairs of rods and a pair of dots, while *pinicola* has a more derived karyotype, resulting from one autosomal fusion and an X-autosomal fusion. Both species are fungivorous and live in areas that are cool and moist. Both species have double length X chromosomes. The courtship behavior of *pinicola* consists of only a few elements, i.e., bobbing of the abdomen, curling the tip of the abdomen under and lunging onto the female. The *flavopinicola* male also exhibits comparable courtship elements but can and usually does display a number of additional elements. *D. pinicola* males engage in a motionless trance-like behavior at the end of the copulatory period, but *flavopinicola* appears to lack this behavior. The basic courtship pattern of *Drosophila* males is to orient upon a female, tap with the fore tarsi and then engage in courtship display. Both *flavopinicola* and *pinicola* will display without the physical presence of a female. Thus

the male display is in reality an advertising ploy that presumably may serve to attract receptive females to him.

In a number of ways, especially in male courtship sequences of the two species, there are unique display elements which are lacking in most *Drosophila* species but are exhibited by a number of Hawaiian drosophilids. For example, the use of both wings in the display, the production and deposition on the substrate of an anal droplet, the elevation of the tip of the abdomen with concurrent lowering of the head, the trance state at the end of the copulation and the performance of the display without the immediate presence of a female. Likewise, the excessively long X chromosomes are similar to those of the Hawaiian species. The female ventral receptacles are similar to those of many Hawaiian species. Most Hawaiian species have dorsal spermathecae that lack an introvert (Throckmorton 1966), but two species, *D. anomalipes* Grimshaw and *D. quasianomalipes* Hardy, which appear to be archaic members of the Hawaiian fauna (Spieth, in press), have spermathecae that are similar to those of *pinicola* and *flavopinicola*. The long life cycle and the restriction to relatively low environmental temperatures (i.e., 18–19°C) are also characteristic of the Hawaiian species.

These evidences lead us to suggest that *pinicola* and *flavopinicola* are the descendants of an ancestral population that was closely related to the ancestor which gave rise to the Hawaiian *Drosophila* fauna.

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## RECENT LITERATURE

THE INSECTS OF VIRGINIA: NO. 7. THE AQUATIC AND SEMI-AQUATIC HEMIPTERA OF VIRGINIA. Marvin L. Bobb. Virginia Polytechnic Institute and State University Research Division Bulletin 87:1-195. 1974.

This portion of the series dealing with Virginia insects includes 97 species in 30 genera among 14 families of Hemiptera. Keys are provided to the families and for the genera and species of each family. A brief diagnostic statement is given for each taxon and additional information on distribution, habitat, and biology is presented for the species. The 20 plates are well-done and should aid in the identification of the species. On the whole, the paper appears well-done and should be useful for studies on aquatic Hemiptera in that region.—EDITOR

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THE INSECTS OF VIRGINIA: NO. 8. THE AQUATIC HYDROPHILIDAE OF VIRGINIA. James F. Matta. Virginia Polytechnic Institute and State University Research Division Bulletin 94:1-44. 1974.

This is cited as being the forerunner of treatments of the aquatic Coleoptera of Virginia. The aquatic Hydrophilidae are presented in similar fashion as other groups in this series. A total of 49 species are discussed with keys for their separation. Although illustrations of adults are lacking, genitalia of several species are figured.